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p21(WAF1) (/Cip1) limits senescence and acinar-to-ductal metaplasia formation during pancreatitis

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Abstract: Trans-differentiation of pancreatic acinar cells into ductal-like lesions, a process defined as acinar-to-ductal metaplasia (ADM) is observed in the course of organ regeneration following pancreatitis. In addition, ADM is found in association with pre-malignant PanIN lesions and correlates with an increased risk of pancreatic adenocarcinoma (PDAC). Human PDAC samples show down-regulation of p21(WAF1) (/Cip1) , a key regulator of cell cycle and cell differentiation. Here we investigated whether p21 down-regulation is implicated in controlling the early events of acinar cell trans-differentiation and ADM formation. p21-mediated regulation of ADM formation and regression was analyzed in vivo during the course of cerulein-induced pancreatitis using wild type (WT) and p21 deficient (p21(-/-)) mice. Biochemical and immunohistochemical methods were used to evaluate disease progression over two weeks of the disease and during a recovery phase. We found that p21 was strongly up-regulated in WT acinar cells during pancreatitis, while it was absent in ADM areas, suggesting that p21 down-regulation is associated with ADM formation. In support of this hypothesis, p21(-/-) mice showed a significant increase in number and size of metaplasia. In addition, p21 over-expression in acinar cells reduced ADM formation in vitro, suggesting that the protein regulates the metaplastic transition in a cell-autonomous manner. p21(-/-) mice displayed increased expression and re-localization of -catenin during both pancreatitis and subsequent recovery phase. Finally, loss of p21 was accompanied by increased DNA damage and development of senescence. Our findings are consistent with a gate-keeper role of p21 in acinar cells to limit senescence activation and ADM formation during pancreatic regeneration.

DOI: https://doi.org/10.1002/path.4440

Posted at the Zurich Open Repository and Archive, University of Zurich
ZORA URL: https://doi.org/10.5167/uzh-98905
Journal Article
Accepted Version

Originally published at:
Grabliauskaite, Kamile; Hehl, Adrian B; Seleznik, Gitta M; Saponara, Enrica; Schlesinger, Kathryn; Zuellig, Richard A; Dittmann, Anja; Bain, Martha; Reding, Theresia; Sonda, Sabrina; Graf, Rolf (2015). p21(WAF1) (/Cip1) limits senescence and acinar-to-ductal metaplasia formation during pancreatitis. Journal of Pathology, 235(3):502-514.
DOI: https://doi.org/10.1002/path.4440

Urodynamic investigations in patients with spinal cord injury: Should the ice water test follow or precede the standard filling cystometry?

Urodynamics in spinal cord injury: timing of ice water test

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ABSTRACT

Objectives: To evaluate whether the ice water test (IWT) should be performed before or after the standard urodynamic investigation (UDI).

Patients & Methods: Two cohorts of patients suffering from neurogenic lower urinary tract dysfunction (NLUTD) due to spinal cord injury (SCI) were matched by lesion level and age. The patients of cohort A (n = 55, retrospective cohort) underwent the IWT before and the patients of cohort B (n = 110, prospective cohort) after standard UDI. The IWT effect on urodynamic parameters has been compared between the two groups using the Mann-Whitney-U test for independent samples. UDI was performed according to Good Urodynamic Practices recommended by the International Continence Society.

Results: The mean age of both cohorts was 49 years. Performing the IWT before versus after standard UDI resulted in a significantly lower maximum cystometric bladder capacity ($p=0.01$), lower incidence of detrusor overactivity ($p=0.017$) and lower maximum detrusor pressure during IWT ($p=0.04$). All other urodynamic parameters assessed demonstrated no significant difference ($p>0.05$).

Conclusions: Our results are in line with findings from animal studies demonstrating a bladder cooling induced gating effect on the micturition reflex volume threshold on the level of sacral interneurons. Since the IWT is an unphysiological investigation that might significantly bias subsequent urodynamics, we suggest that the IWT should not precede more physiological standard UDI.

KEY WORDS

lower urinary tract dysfunction; diagnostic tests; urodynamics; ice water test; bladder cooling reflex; spinal cord injury

INTRODUCTION

The bladder cooling reflex is a spinal reflex that can be urodynamically investigated using the ice water test (IWT), i.e. rapid infusion of cold saline into the bladder, which was first described 1957 by Bors and Blinn to differentiate between a lesion of the upper (positive bladder cooling reflex) or lower (negative bladder cooling reflex) motoneuron in spinal cord injury (SCI) patients [1]. Studies in cats indicated that the bladder cooling reflex is a C-fibre mediated lower motoneuron segmental reflex and that unmyelinated C-fibres are associated with specific cold receptors of the transient receptor potential family [2-4]. Clinical investigations and immunohistochemical studies provided evidence that the mechanism of the bladder cooling reflex might be also true for humans [5].

Since C-fibres seem to play a role in the pathomechanism of lower urinary tract symptoms (LUTS) not only in neurological patients [6-9], the clinical relevance of the IWT might even reach beyond the simple differentiation between an upper and lower motoneuron lesion.

Geirsson et al. elaborated on the use of the IWT in humans and suggested to infuse 100 mL saline <10°C at a speed of 100 to 300 mL/s as standard for optimized bladder cooling reflex assessment and proposed a detrusor contraction in response to bladder cooling of >30cmH2O as positive IWT result [10].

However, in daily urodynamic practice, it is still unclear, if the IWT should be performed before or after the standard urodynamic investigation (UDI) and in how far both investigations affect each other. Thus, we aimed to investigate the outcome of the IWT and its effect on urodynamic parameters when performed before or after standard UDI.

PATIENTS AND METHODS

This study was approved by the local ethics committee (Kantonale Ethikkommission Zürich) and conducted at the Spinal Cord Injury Centre, Balgrist University Hospital, Zürich, Switzerland.

The study comprised two independent cohorts: Cohort A (retrospective data, IWT before UDI): Urodynamic data from SCI patients with neurogenic lower urinary tract dysfunction (NLUTD) investigated at our center from January 2001 until September 2010 were reviewed. During this time period the IWT was exclusively performed prior to the standard UDI.

Cohort B (prospective data, IWT after UDI): From October 2010 to June 2012, SCI patients with NLUTD undergoing standard UDI and IWT were eligible for study participation. To be included, patients had to provide written informed consent. In this study part, the IWT was exclusively performed after standard UDI.

Exclusion criteria for both cohorts were current urinary tract infection, pregnancy, intradetrusor onabotulinumtoxinA injections within the last year before UDI and patient age < 18 years old.

In cohort B, 242 patients could be prospectively enrolled and were matched by lesion level and age with 57 patients of cohort A. Propensity score matching resulted in 110 patients in cohort B and 55 patients in cohort A.

All methods, definitions, terms, and units used in this study are in accordance with the standards recommended by the International Continence Society [11].

Urodynamic investigation (UDI)

UDIs were done in accordance to good urodynamic practices recommended by the International Continence Society [12]. A multichannel urodynamic system (Sedia®, Givisiez, Switzerland) was applied for all measurements. Pelvic floor

electromyographical data were recorded via two perineal surface electrodes. Filling sensations were documented when applicable. Standard UDI was performed using body warm saline and a filling speed of 20-30 mL/min. The IWT was conducted with 4°C saline and a filling speed of 100 mL/min.

Outcome measures

From storage phase of standard UDI: Maximum cystometric bladder capacity (mL), bladder volume at first detrusor overactivity (mL), incidence of detrusor overactivity (%), maximum detrusor pressure (cmH_2O), and bladder compliance during ($\Delta mL/\Delta cmH_2O$).

From IWT: maximum detrusor pressure (cmH_2O).

Statistical analyses

Data were approximately normally distributed and are shown as mean ± standard deviation (SD). To limit the observational character of the study we analyzed a propensity matched sample. For the computation of the propensity score, age and lesion level were included into a logistic regression with the IWT performed before or after the standard UDI as dependent variable. The validity of logistic regression was assessed using the Hosmer-Lemeshow test. Propensity matching was performed using the package Matching in R [13]. Cohort A and B were matched with a proportion of 1:2. Patients of cohort A and B were compared using the Mann-Whitney-U test. Statistical analyses were performed applying IBM® SPSS® version 22 (IBM® Armonk, USA). A p-value <0.05 was considered statistically significant.

RESULTS

The patients' characteristics of cohort A and B are shown in table 1.

Table 2 summarizes the outcome parameters of both cohorts. Performing the IWT before versus after standard UDI resulted in a significantly lower maximum detrusor pressure during IWT ($p=0.04$) and a lower maximum cystometric bladder capacity ($p=0.01$), lower incidence of detrusor overactivity ($p=0.017$) during subsequent standard UDI and lower maximum detrusor pressure during IWT ($p=0.04$). All other urodynamic parameters assessed demonstrated no significant difference ($p>0.05$).

DISCUSSION

Our current findings suggest that performing the IWT prior to the standard UDI has effects on the outcome of the UDI, namely a decrease in maximum cystometric bladder capacity and bladder volume at first detrusor overactivity. Jiang CH et al. assumed based on findings from animal studies with cats that the dynamic cooling reflex is a C-fibre mediated reaction evoked by rapid cooling of the bladder, which leads to a reflex discharge in bladder preganglionic neurons [14, 15]. Thereafter the cold evoked efferent activity persists for several tens of seconds and leads to a reduction of the threshold volume of the A-delta mechanoreceptor driven micturition reflex [14, 15]. In line with the observations of Jiang et al. we found a reduced volume threshold for detrusor overactivity but no changes in compliance or maximum detrusor pressure, indicating that the A-delta mechanoreceptor driven micturition reflex itself remains unchanged but is gated at a lower threshold volume [15].

Jiang et al. suggested that this gating of the micturition reflex occurs rather on a spinal level through preganglionic interneurons than via the pontine micturition center (PMC) due to the observation that bladder C- and A-delta fibre mediated reflexes are modulated in parallel by a spinal enkephalinergic recurrent inhibitory mechanism [15].

This recurrent inhibition, which has been demonstrated to originate from axon collaterals of bladder preganglionic neurons [16, 17], is supposed to act at spinal interneurons prior to the preganglionic lower urinary tract (LUT) neurons [15, 18].

The PMC in contrast induces the micturition reflex via one long descending pathway to the sacral spinal cord where the reflex is further mediated by segmental interneurons as an all-or-none response [19]. Only the latter reflex enhanced component but not the descending component from the PMC has been demonstrated to be susceptible to modulation by recurrent inhibition [15]. This would also explain why the bladder cooling induced modulation of the micturition reflex can be also observed in our SCI study population.

Van Meel et al. observed similar effects during repeated IWT with an increase of positive IWT results with each repetition [20]. Although repeated IWT in conjunction with LUT current perception threshold measurement can be helpful to identify unsuspected neurological pathologies in patients with assumed idiopathic LUTS [20], the IWT still remains an unphysiological stimulation that can significantly bias subsequent investigations such as standard UDI. Since the standard UDI aims to reflect the daily LUT function as close and physiological as possible, we recommend - based on our findings - to perform the IWT after the standard UDI to obtain unbiased and consequently comparable UDI results. If in patients with NLUTD repeated standard UDIs as recommended previously [21] do not reveal any findings correlating with the daily symptoms, an IWT might be used as provocation test to reveal masked pathologies such as detrusor overactivity but after the standard UDI.

Considering that the human bladder seems to be innervated by A-delta and C-fibres only [22], urodynamic testing of both fibre types offers the possibility to more

comprehensively assess the underlying pathology of the clinical symptoms. This might be especially relevant since cold sensitive transient receptor potential channels, i.e. TRPM8, and cold sensitive C-fibers have been specifically associated with lower urinary tract dysfunction [7, 22, 23].

Due to their high intravesical pressure thresholds, C-fibers are in contrast to Ad-fibers considered less relevant for normal, gradual development of bladder sensations such as first sensation of filling, first desire to void and strong desire to void [22]. However, C-fiber mechanosensitivity might change in response to chemical or thermal stimuli or alterations in neuronal control such as spinal cord injury [24]. These nociceptive properties of C-fibers might be related to the finding that they express various receptors including those of the transient receptor potential family [22]. In conjunction with their abundant distribution within the LUT which accounts for approximately two thirds of bladder afferent neurons in rats [25], C-fibers represent a relevant sensory component of the LUT that, in case of aberrant functioning, might be accounted for several irritative symptoms of which some are clinically summarized as overactive bladder (OAB) [26]. Hence, assessment of such C-fibers in patients might improve our understanding on LUT symptoms [6, 27].

Although there is a lack of validated standardization on performing and interpreting the IWT, the description by Geirsson et al. [10] and the review by Al-Hayek et al. [28] provides a useful first recipe to perform the IWT. Since the IWT in our study has been performed in the same manner in all patients, a comparison between our cohorts is valid.

There are limitations of this study that should be considered: The study design was not fully prospective, rather one cohort consisted of retrospective data only. The presented results are based on findings in patients with NLUTD due to SCI. Thus, our findings cannot be generalized beyond this group.

In conclusion, this is the first study to evaluate the effect of the IWT on the standard UDI in SCI patients with NLUTD. Since the IWT is a rather unphysiological investigation that might significantly bias subsequent urodynamics, we suggest that the IWT should not precede more physiological standard UDI.

ACKNOWLEDGEMENT

This study was funded by the Swiss Continence Foundation (www.swisscontinencefoundation.ch) and the Clinical Research Priority Program NeuroRehab of the University of Zürich. The Swiss Continence Foundation and the Clinical Research Priority Program NeuroRehab of the University of Zürich had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

CONFLICT OF INTEREST STATEMENT

None of the authors has a conflict of interest related to the submitted work.

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TABLES AND THEIR LEGENDS

Table 1. Baseline characteristics of the patients of the two cohorts. Matching of subjects between Cohort A and B was performed using propensity score analysis with a proportion of 1:2.

	Cohort A (= IWT before FC) mean ±SD (range)	**Cohort B** (= IWT after FC) mean ±SD (range)	
No. of patients (all with NLUTD due to SCI)	55	110	
Age in years, mean ± SD (range)	49 ± 15 (20 - 85)	49 ± 15 (20 - 81)	p = 0.966
Gender distribution			
Female, n (%)	25 (45)	19 (17)	p < 0.001
Male, n (%)	30 (55)	91 (83.8)	
Level of lesion			
Cervical, n (%)	17 (33)	38 (34)	p = 0.906
Thoracic, n (%)	35 (67)	75 (66)	

Table 2. Outcome parameters comparing IWT performed before and after standard urodynamic

	Cohort A (= IWT before FC) mean ±SD (range)	**Cohort B** (= IWT after FC) mean ±SD (range)	
maximum cystometric bladder capacity [mL]	444 ± 158 (40 - 800)	611 ± 276 (110 - 1400)	p=0.01
bladder volume at first detrusor overactivity [mL]	288 ± 133 (25 - 600)	360 ± 190 (35 - 1060)	p=0.059
incidence of detrusor overactivity	71 % (n = 39)	86 % (n = 95)	p=0.017
maximum detrusor pressure during storage phase of standard UDI [cmH_2O]	32 ± 15 (10 - 67)	39 ± 20 (11 - 99)	p=0.102
maximum detrusor pressure during IWT [cmH_2O]	36 ± 31 (2 - 127)	43 ± 26 (0 - 116)	p=0.04
bladder compliance [$\Delta mL/\Delta cmH_2O$]	46 ± 26 (13 - 150)	56 ± 51 (9 - 350)	p=0.597